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Transcriptome Analysis of Ido1 Knock-out Mice with Dextran Sulfate Sodium-induced Colitis

Ido1 Knock-out 마우스를 이용한 Dextran Sulfate Sodium 유도 대장염 모델에서의 전사체 분석

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Abstract

Transcriptome Analysis of Ido1 Knock-out Mice with Dextran Sulfate Sodium-induced Colitis

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Tryptophan involves in a range of biological processes including protein and biogenic nitrogen-compounds synthesis. It is metabolized to kynurenine by indoleamine 2,3-dioxygenase1 (IDO1) that is the first rate limiting enzyme. IDO1 expresses ubiquitously in the body with heightened expressions in intestinal tissues. To examine the function of IDO1 in colon, transcriptome analysis using microarray was performed in both Ido1 knock-out ($Ido1^{-/-}$) mice and wild type ($Ido1^{+/+}$) mice. Differentially expressed genes in comparison of $Ido1^{-/-}$ and $Ido1^{+/+}$ mice were categorized based upon

their biological functions. Gene set enrichment analysis showed that inflammatory response was the most significant category which was modulated by IDO1 gene. This observation prompted us to study the function of IDO1 in inflammatory bowel disease mouse model. In DSS-induced ulcerative colitis model, the disease was more severely developed in $Ido1^{+/+}$ mice compared with $Ido1^{-/-}$ mice. Total RNAs of inflamed colon tissues from both $Ido1^{+/+}$ and $Ido1^{-/-}$ mice were applied to microarray in order to find the significant signaling pathways affected by Ido1 deficiency. TLR signaling and NF-kB signaling were turned out to be responsible for generating the difference in disease progression between those two genotypes. Dramatic changes in TLR signaling and NF-kB signaling resulted in substantial changes in expressions of many pro- and anti-inflammatory cytokines and chemokines. These findings suggest that IDO1 play roles in producing inflammatory responses and modulating transcriptional networks during colitis development.

Key words: tryptophan, IDO1 (Indoleamine 2,3-dioxygenase1), colitis, transcriptome, inflammatory response

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List of Abbreviations

ABL1, abelson murine leukemica viral oncogene homolog 1

ACMSD, aminocarboxymuconate semialdehyde decarboxylase

AFIMD, arylformamidase

APC, antigen presenting cell

DC, dendritic cell

HAAO, 3-hydroxyanthranilate 3,4-dioxygenase

IDO1, indolemaine 2,3-dioxygenase 1

IDO2, indolemaine 2,3-dioxygenase 2

IFN-r, interferon gamma

IL-1β, interleukin 1 beta

IL-7, interleukin 7

IL-18, interleukin 18

IKK, I kappa B kinase

IRF 5, interferon regulatory factor 5,

IRS-1, insulin receptor substrate 1

JNK, c-Jun N-terminal kinase

KMO, kynurenine 3-monooxygenase

KYNU, kynureninase

MCP-1, monocyte chemoattractant protein 1

MMP3, matrix metalloproteinase 3

MUC1, mucin 1

MYD88, myeloid differentiation primary response gene 88

NAD, nicotinamide adenine dinucleotide

NADSYN, nicotinamide adenine dinucleotide synthetase 1

NF-kB, nuclear factor kappa-light chain-enhancer of activated B cells

NMNAT, nicotinamide nucleotide adenylyltransferase

SERT, serotonin receptor

TAP1, transporter associated with antigen processing 1

TDO, tryptophan 2,3-dioxygenase

TNF, tumor necrosis factor

TLR, toll like receptor

TPH, tryptophan hydroxylase

P38 MAPK, p38 mitogen activated protein kinase

QPRT, quinolinate phosphoribosyltransferase

Ⅰ. Introduction

The metabolism of amino acids has been the subject of numerous interesting studies over the last years. Among the 20 amino acids that constitute proteins, tryptophan is especially noteworthy, because for many organisms (including humans), tryptophan is an indispensable amino acid that should only be up-taken through diet. Tryptophan is the precursor of multifarious aspects of nutrition and metabolism. Tryptophan is involved with a variety of processes including protein synthesis and is an important biochemical precursor producing biogenic nitrogen-containing compounds such as serotonin (a neurotransmitter), melatonin (a neurohormone), tryptamine, and tryptophan metabolites similar to kynurenines.

In mammals, tryptophan is catabolized along in variety routes. Tryptophan primarily contributes in two pathways: the synthesis of nicotinamide adenine dinucleotide (NAD^+), a coenzyme importantly required to energy metabolism via the kynurenine pathways, and synthesis of the putative transmitter serotonin, an important neuromediator regulating gastrointestinal functions, mood, appetite, and sleep via the serotonin signaling pathway. Approximately 95% of serotonin content in the body is found in the intestine (Levin et al., 2013). The conversion of tryptophan to serotonin occurs by tryptophan hydroxylase (TPH) and a small portion of serotonin is

further converted into melatonin. Majority of tryptophan is known to be metabolized by kynurenine pathway in which tryptophan degrades through a series of metabolic reactions (Rios-Avila et al., 2013). Both key enzymes, indoleamine 2,3-dioxygenase1 (IDO1, also known as tryptophan pyrrolase) and tryptophan 2,3-dioxygenase (TDO, also known as tryptophan oxygenase), catalyze the oxidative cleavage of L-tryptophan (L-Try) to N-formyl-L-kynurenine in the first and rate-limiting step of the kynurenine pathway, depending on tissue and cell types (Stavrum et al., 2013). In 1937, TDO was first found to be present in the liver, catalyzing the first step of the kynurenine pathway (Miller et al., 2004). In fact, TDO operates mainly in the liver (Le Floch et al., 2011) and its expression is induced by tryptophan itself or by cortisol, which is secreted in response to stress. On the other hand, in the same year, an enzyme was detected in the extra-hepatic tissues that catalyze the same oxidative rings' cleavage of tryptophan named IDO1 in the rabbit intestine (Takikawa et al., 2005). It has been reported that IDO1 was ubiquitously distributed in extra-hepatic tissues such as the colon (primarily distal colon) (Matteoli et al., 2010), small intestine (primarily terminal ileum) (Mellor et al., 2004), brain (Kwidzinski et al., 2005), spleen (Boasso et al., 2007), lung (Romani et al., 2008), and in cells including macrophages, dendritic cells (DCs) (Mellor et al., 2004). IDO2, a paralogue enzyme of IDO1, was recently discovered (Merlo et al., 2014). Biologically,

IDO2 functions like IDO1 in tryptophan metabolism, but is expressed in kidney, liver (Fatokun et al., 2013), and reproductive system (Fukunaga et al., 2012), showing a different tissue distribution to that of IDO1 (Murakami et al., 2013) as well as very low activity.

In the last decade, the IDO1-initiated tryptophan metabolism has been attracting more interest because of its great potential effect on various physiological functions. The first discovery of the role of IDO1 expressed in the placenta, whereby maternal T cell mediated immunity during gestation, indicated that IDO1 can regulate T cell activity through tryptophan catabolism (Munn et al., 1998). They administered 1-methyl-tryptophan (1-mT), which inhibits IDO1 activity, to pregnant mice and found IDO1 suppressed T cell proliferation. In addition, several studies have shown one of the biological functions of IDO1 is to act as an immune-suppressor mediated primarily by T cells in the adaptive immune system. Terness et al. (2002) showed that up-regulated IDO1 increased production of tryptophan metabolites, which generated cell-cycle arrest of T cell, further promoted apoptosis of T cell and induced regulatory T cell differentiation. More recently, a new immune escape model has been proposed for tumors based on the interactions of the IDO1 function and T cell response. High IDO1 expression by tumor cells can contribute to recruitment of antigen-presenting cells (APCs) for their immune tolerance and facilitate tumor

escape (Balachandran et al., 2011). Likewise, treatment with an IDO1 inhibitor has also been reported to reduce the volume of tumors, suggesting that the competitive inhibitor of IDO1 may be a novel molecule for tumor immunotherapy. Despite the given the immune-modulatory role of IDO1, the mechanism by which IDO1 mediates immune tolerance is a field of active investigation and remains somewhat controversial.

Aim of this study

In this study, we performed for the first time a whole genome transcriptional profile analysis to investigate the role of IDO1 on tryptophan metabolism by using Ido1 knock-out mice, leading to the identification of new molecules and describing functionally distinct molecular mechanisms regulated by IDO1 expression. This first large-scale profiling may enhance understanding of the IDO1 biological activity, and identify novel potential target genes to be exploited as candidates related to tryptophan metabolism. Moreover, we aimed to elucidate pathophysiological mechanisms by which IDO1 play roles in colitis development through looking at transcriptional networks in DSS-induced colitis model mice.

Ⅱ. Materials and Methods

Animals

Ido1$^{-/-}$ mice of the C57BL/6J background were obtained from The Jackson Laboratory (Bar Harbor, Maine, USA). Ido1$^{-/-}$ mice were crossed with C57BL/6J (The Jackson Laboratory) to generate the Ido1$^{-/-}$ and Ido1$^{+/+}$ offspring used in this study. Genotypes of knockout mice were verified via PCR typing. The mice used were 6–12 weeks old and weighted 18–23g. Age-matched female mice were used in experiments. Animals were individually housed in plastic cages under specific pathogen-free conditions at the Center for Animal Resource Development of Seoul National University College of Medicine (Seoul, Korea). The room was controlled with constant temperature (25 ± 2ºC), humidity (55 ± 10%), and a 12-hour dark/light cycle. All animal experiments were carried out in accordance with guidelines of the Animal Ethics Committee of Seoul National University (Seoul, Korea).

Induction of Colitis

To generate an acute colitis experimental model, DSS (molecular mass 36,000-50,000Da; MP Bio-chemicals, LLC, Illkirch, France) was added to drinking water at concentrations of 1% (n=5) or 2% (n=4) (w/v), which was given *ad libitum* for 7 days. Control mice received drinking water without DSS (n=3). The subsequent course of colitis development was evaluated by monitoring the daily weight changes. Colitis severity also was scored by evaluating the clinical disease activity through daily observing of the following parameters: weight loss (0 points = No weight loss, 1 points = 5–10% weight loss, 2 points = 11–15% weight loss, 3 points = 16–20% weight loss, 4 points = >21% weight loss); stool consistency (0 points = normal and well formed, 2 points = very soft and unformed, 4 points = watery stool); and bleeding stool score (0 points = normal color stool, 2 points = reddish color stool, 4 points = bloody stool). The disease activity index (DAI) was calculated by the combined scores of weight loss, stool consistency, and bleeding, as detailed in **Table 1.** All parameters were scored from day 0 to day 7.

Table 1. Disease Activity Index (DAI) Criteria[a]

Weight loss	Stool consistency	Fecal bleeding
0 = No weight loss	0 = Normal and well formed	0 = Normal color stool
1 = 5-10% weight loss		
2 = 11-15% weight loss	2 = Very soft and unformed	2 = Reddish color stool
3 = 16-20% weight loss		
4 > 21% weight loss	4 = Watery stool	4 = Bloody stool

a. Cooper H, Murthy SNS, Shah RS, Sedergran DJ: **Clinicopathologic study of dextran sulfate sodium experimental murine colitis.** *Laboratory Investigation; a Journal of Technical Methods and Pathology.* 1993; **69**(2):238-249.

Tissue Collection

At the 8th day after DSS-colitis induction, mice were sacrificed and entire colon was quickly removed, cut open lengthwise, and gently flushed with sterile phosphate-buffered saline (PBS) to remove any traces of feces. Colon segments were immediately frozen in liquid nitrogen and stored at -80 °C for subsequent total RNA extraction. For histological analysis, colon segments were fixed in 10% buffered formalin phosphate and stored at room temperature until histological evaluation of inflammation.

Histological Analysis of Colitis

Routinely processed, paraffin-embedded sections of the colon samples were prepared and stained with hematoxylin and eosin (H&E) for histological grading. Histological scores, including severity of colitis, were evaluated in a blinded manner as previously described by Laroui et al. (2012). Grades were evaluated from 0 – 4 for the following three criteria: severity inflammation (0, rare inflammatory cell in the lamina propria; 1, increased inflammatory cell in the lamina propria; 2, confluence inflammatory cell extending into the submucosa; and 3, transmural extension of the inflammatory infiltrate), damage (0, none; 1, loss of the basal 1/3 of the crypt; 2, loss of the basal 2/3 of the crypt; 3, loss of the entire crypt but intact epithelial cell; and 4, loss of the entire crypt and of the surface epithelial cell), extension (0, none; 1, focal; 2, lesion involving 1/3 of the intestine; 3, lesion involving 2/3 of the intestine; and 4, lesion involving the entire intestine). Scores for each criterion were added to give an overall inflammation score for each sample of 0-11. The histological grades were determined for each section, and the sum of the grades was reported as the inflammation score for each mouse. Two histopathologists blindly assessed the level of colitis.

Microarray Hybridization and Scanning

For microarray hybridization, total RNA was isolated by homogenizing the samples from the colon tissue and purified using a DNA-free RNA isolation kit (RNAqueous-4PCR kit; Ambion, Austin, TX, USA) in accordance with the manufacturer's instructions. Total RNA integrity and quantity were assessed with a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) before the microarray experiments. Only total RNA with an OD 260/280 ratio > 2.0 was used for microarray hybridization. RNA samples were first amplified for array analyses using the Illumina TotalPrep RNA Amplification Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. Briefly, 500ng of total RNA was used to prepare labelled cRNA with overnight incubation according to the manufacturer's protocol. The quality and quantity of the labelled cRNA were monitored using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Amplified cRNA (1.5g) was hybridized on MouseWG-6 Expression BeadChip arrays, containing more than 45,200 well-annotated Ref transcripts, according to the manufacturer's standard protocol. The arrays were then scanned on a BeadArray Reader (BeadStation 500G Instrument, Illumina Inc.), and Spot images identification and quantification were obtained by the Genome Studio software v1.0.2. (Illumina Inc.).

Identification of Significant Genes

The raw data was log-transformed, and normalized by quantile normalization method using Genome Studio software (Illumina Inc.). Significant difference between two genotypes in each dose (0% [baseline], 1% treatment, 2% treatment), differences between dose response effect in each genotype, and difference between genotype x dose interaction were identified using ANOVA test ($p < 0.05$) on log2-transformed normalized intensities using by Partek® Genomics Suite software v6.3 (Partek, St Louis, MI) (http://www.partek.com/partekgs). Transcripts with more than 2-fold differential expression and a false discovery rate (FDR) < 0.01 were selected for each specific comparison analyzed.

Functional Enrichment and Clustering Analysis

Functional categorization analysis was performed based upon gene ontology consortium (GO). Gene Set Enrichment Analysis (GSEA) was done (http://www.broadinstitute.org/gsea/index.jsp) to examine the significance of each functional category classified by GO. Hierarchical clustering analysis was carried out with Genesis software v1.7.5 (Sturn et al., 2002) using the Pearson correlation distance matrix with average linkage algorithm.

Statistical Analysis

Data were expressed as the mean ± SEM. Statistical significance (p value < 0.05) was evaluated either by unpaired student's t test between two groups or two-way analysis of variance (ANOVA) to compare multiple groups using dose and genotype as factors. When appropriate, one-way ANOVA followed by the Duncan's multiple-range test was used for post hoc analysis to determine differences between groups. To maintain consistency, unpaired student's t test or two-way ANOVA test was used for all comparisons. Statistical analyses were performed using Graph Pad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) and SAS Enterprise Guide 6.1 (SAS Institute Inc., Cary, NC, USA). For gene analyses, significances for functional enrichment of specific genes were determined by a right-tailed Fisher's exact test as the negative log of the probability that the number of focus genes is not due to random chance.

Ⅲ. Results

1. Identification of Differentially Expressed Genes in Ido1$^{-/-}$ Mice

As the first attempt to identify targets of IDO1, which catalyzes the first and rate-limiting step of tryptophan degradation, microarray analysis was performed using by Ido1$^{-/-}$ and Ido1$^{+/+}$ mice. Principal-component analyses (PCA) confirmed that Ido1$^{-/-}$ mice were readily distinguished from Ido1$^{+/+}$ mice, as shown in **Figure 1**. To define those genes whose expression distinguishes Ido1$^{-/-}$ from Ido1$^{+/+}$, differential gene-expression analysis was performed using a stringent cutoff (> 2-fold change, 1% false discovery rate [FDR]), which identified a total of 102 (37 up, 65 down) genes (**Figure 2**).

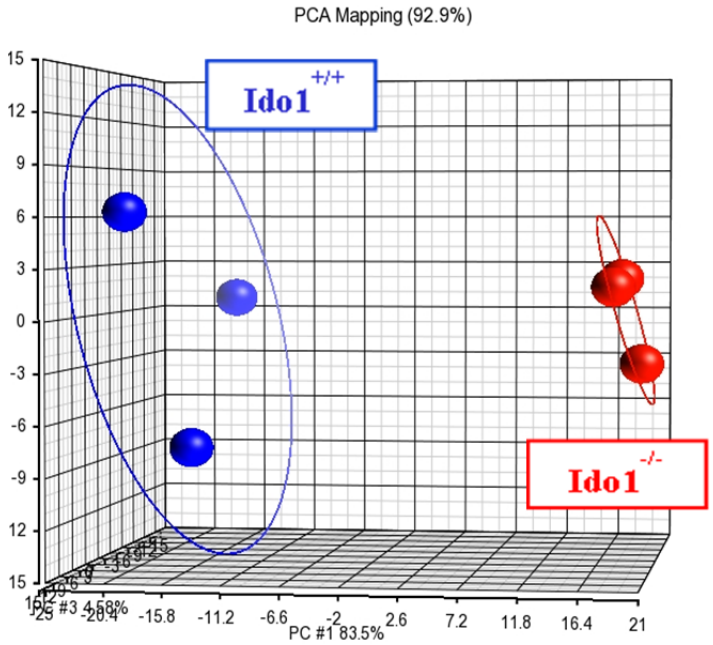


Figure 1. PCA (Principal-component analysis) Plot of Ido1$^{-/-}$ *vs.* Ido1$^{+/+}$ Mice
3-D view of PCA scores plot of Ido1$^{-/-}$ group (n=3) *versus* Ido1$^{+/+}$ group (n=3). Groups are shown by different colors and dots represent individual strains. Blue spots represent Ido1$^{+/+}$ group and the red spots represent Ido1$^{-/-}$ group. On a 3D-PCA plot of Ido1$^{-/-}$ group can be clearly distinguished from Ido1$^{+/+}$ group. Blue spots, Ido1$^{+/+}$ group ; Red spots, Ido1$^{-/-}$ group

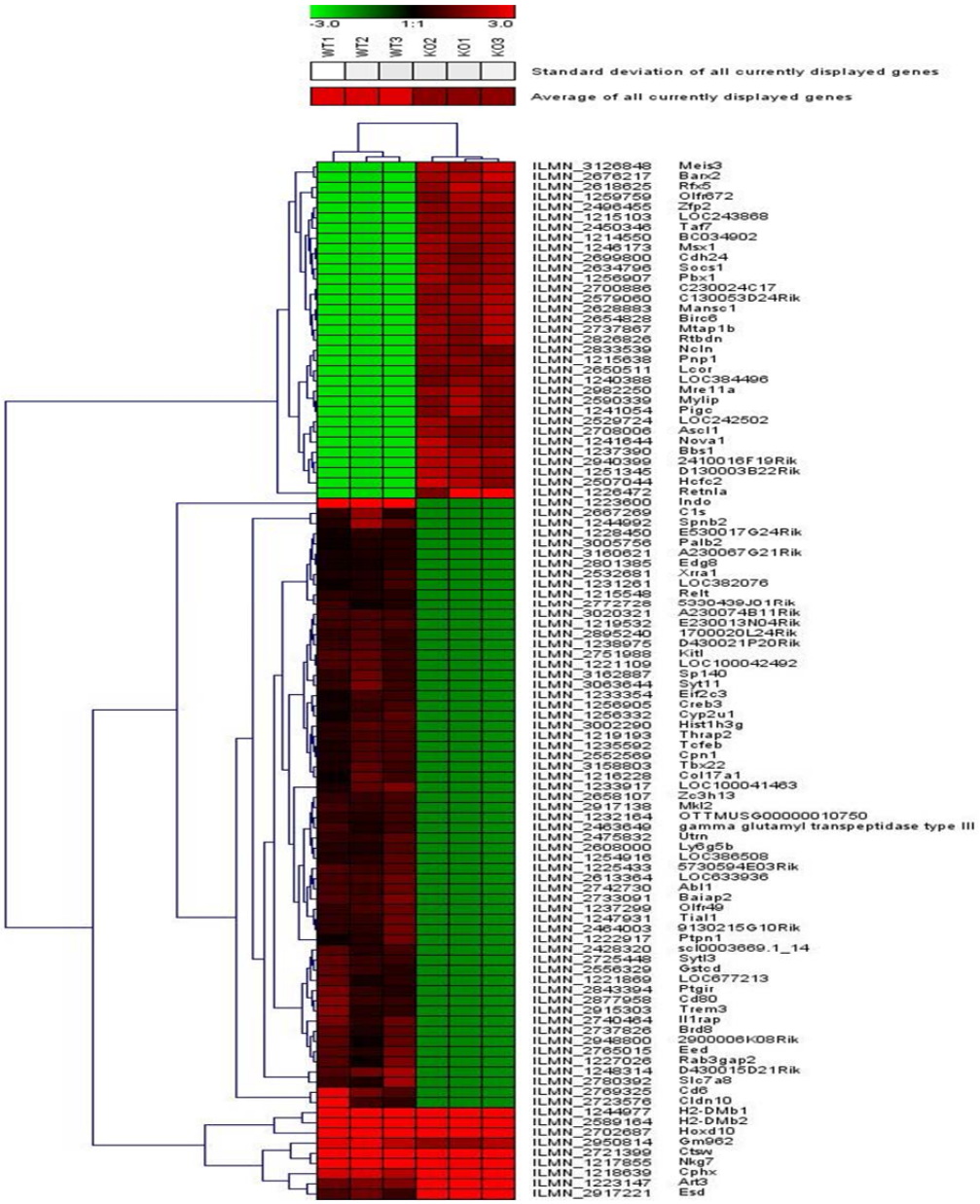


Figure 2. Heat Map of Differentially Expressed Genes in Comparison of Ido1$^{-/-}$ and Ido1$^{+/+}$ Mice

Hierarchical clustering and heat map of up- or down- regulated genes that are differentially expressed (> 2-fold, 1% [FDR]) by IDO1. The 3 samples on the left are Ido1$^{+/+}$ group controls and the 3 samples on the right are Ido1$^{-/-}$ group indicated on each one individaully. The genes differentially expressed are listed from top to bottom along the right edge of the heat map. Red indicates high relative expression and green indicates low expression of genes as shown in the scale bar.

Among the genes regulated by IDO1, we focused initially on those gene expressions associated with tryptophan metabolism. As expected, we confirmed that differentially expressed genes involved in a series of gene encoding enzymes downstream of IDO1, which were referred to as the kynurenine pathway of tryptophan degradation through a series of metabolic reactions and serotonin signaling pathway of the conversion of tryptophan to serotonin (**Figure 3A**). Only five of the genes, *Ido1, Ido2, Kmo, Haao*, and *Alb1*, showed significantly (2-fold greater) differential expression on the two groups (**Figure 3B**). Interestingly, IDO1 deficiency not only reduced the expression of genes associated in NAD^+ biosynthesis pathway (kynurenine pathway) but also increased the expression of genes related to serotonin signaling pathway, including *Tph1, Ddc, and Slc6a4* via negative feedback mechanism (**Figure 3C**).

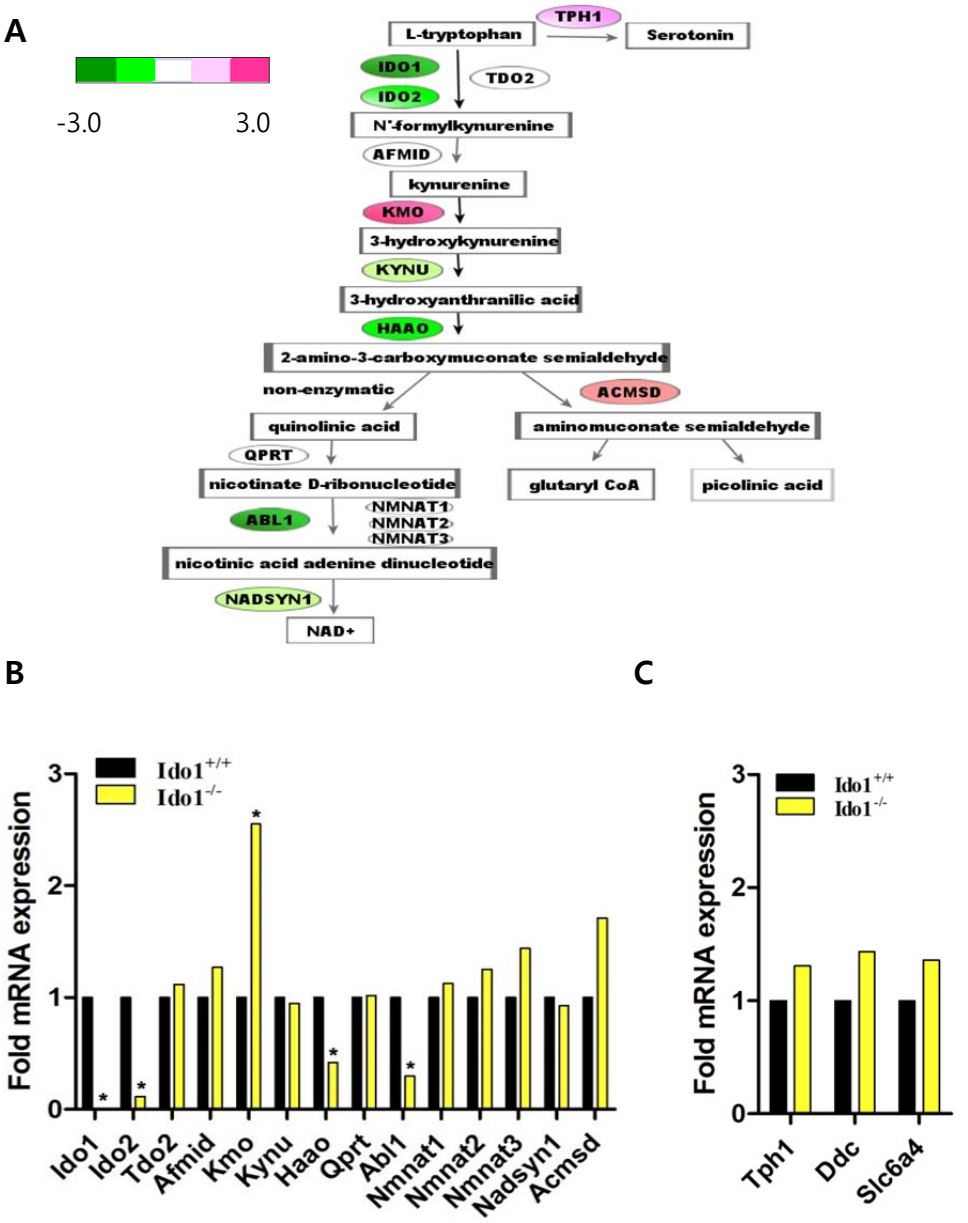


Figure 3. The mRNA Levels of Genes Related to Tryptophan Metabolism

(A) The major metabolic pathway of tryptophan. (B and C) Differential expression of a selection of genes from (A) in the colon tissue was determined by quantification of fold mRNA in unchallenged Ido1$^{-/-}$ and Ido1$^{+/+}$ littermates where the values for Ido1$^{+/+}$ mice were set 1.0. Bar with * indicate significant difference at $P < 0.05$ by unpaired student's t test or > 2-fold at least on one comparison. (B) Expression of genes related kynurenine pathway. (C) Expression of genes related serotonin signaling pathway.

To establish which pathways were the most selectively dysregulated in $Ido1^{-/-}$ mice, we applied a set of functional databases (ingenuity pathway analysis (IPA)) to the list of differentially expressed genes. Our analysis revealed a strong enrichment for genes associated with inflammatory response, suggesting that IDO1 is a key molecule mediating inflammatory response (**Figure 4**). Similarly, gene set enrichment analysis (GSEA) also highlighted that genes associated with inflammatory response are differentially expressed between $Ido1^{-/-}$ and $Ido1^{+/+}$ (FDR < 0.01) (**Figure 5**). In addition, several genes, including H2-dmb1 and H2-dmb2, were found to be down-regulated (more than two-fold significantly differentially expressed), and relative gene expression of Hoxd10 (Homeobox D10), which is a transcription factor that induces cell differentiation such as epithelial cell, was higher in $Ido1^{-/-}$ mice compared to controls. These data suggest that IDO1-deficiency helps re-establishing healthy colon epithelia and has a protective effect of inflammation without inflammatory stimuli. Such findings compelled us to examine how IDO1 contributes to colonic inflammation, as modeled by DSS colitis

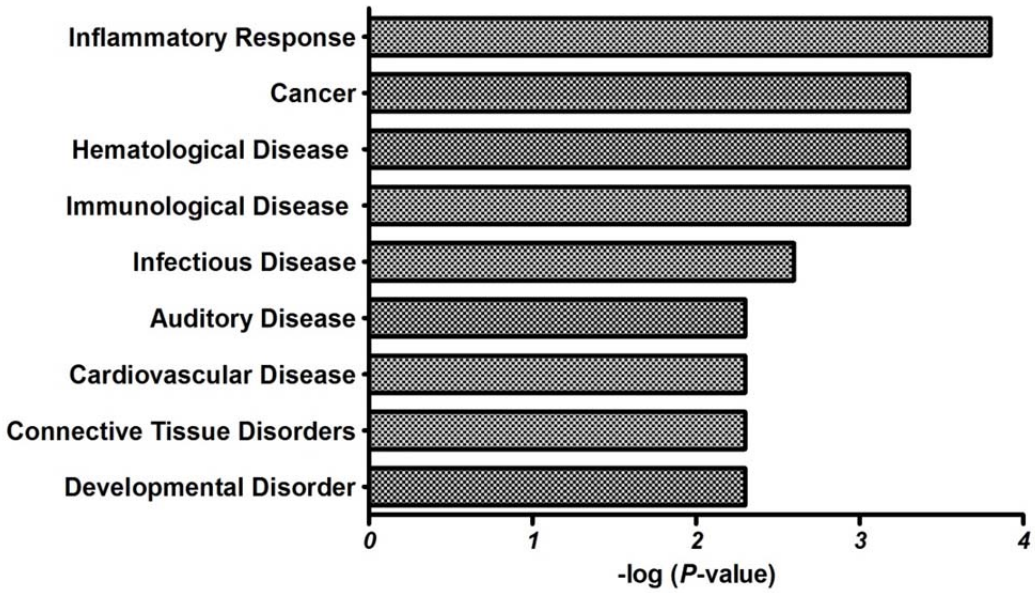


Figure 4. Functional Categories by IPA

Canonical pathway significantly detected in Ido1$^{-/-}$ mice compared to Ido1$^{+/+}$ mice. Statistical significance of pathway modulation was calculated via a right-tailed Fisher's exact test in Ingenutity Pathway and represented as –log p value ($p < 10^{-4}$ Fisher's exact test). A larger value on the x axis indicates a higher degree of significance, i.e., a smaller *p* value.

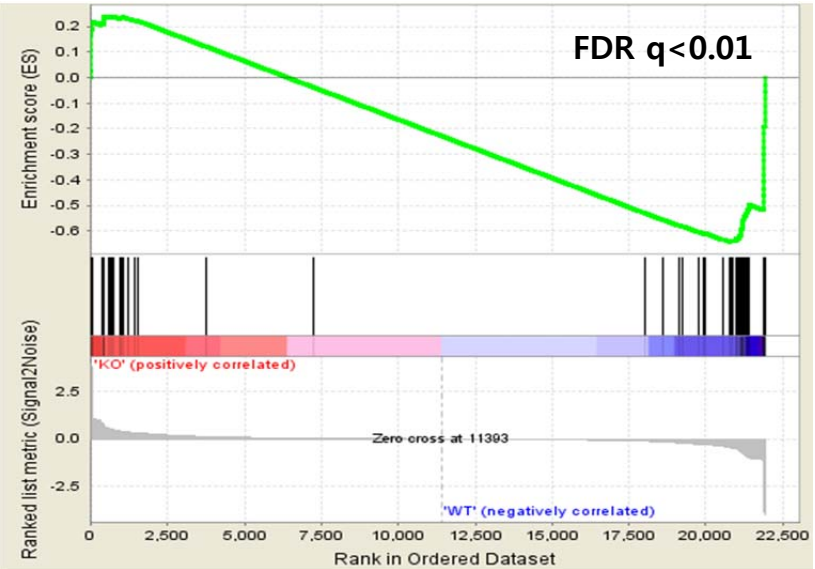


Figure 5. GSEA (Gene Set Enrichment Analysis) of Data Sets

We performed gene-set-enrichment analysis (GSEA) to determine whether the filtered gene list from Ido1$^{-/-}$ mice *vs.* Ido1$^{+/+}$ mice showed specific enrichment in the inflammatory response in the rank-based analysis. Rank of 102 genes in our data sets ordered by expression level with enrichment plots for the up-regulated and down-regulated genes. ES (Enrichment Score) is a value that represents how well the gene set is enriched within the selected gene list. The FDR q value < 0.01 for specific enrichment of the gene set is as indicated. The leading edge analysis of our microarray data identified that the gene is highly correlated with inflammatory response.

2. IDO1 Leads to Increased Susceptibility to Experimental DSS-induced Colitis

Based on the finding that IDO1 affects inflammatory response, we sought to define the contribution of IDO1 to colitic disease. We used an established murine model of colitis induced by oral administration of DSS, a reagent for mucosal epithelial cells that disrupts barrier function (Strober et al., 2002). Responses of Ido1 knock-out mice were compared to those of their littermate wild-type controls. A DSS concentration of 2% (w/v) in the drinking water for 7 days is known to induce strong colitis, but we need to identify difference of both genotypes in mild colitis. For this reason, $Ido1^{-/-}$ and $Ido1^{+/+}$ mice were given either 1% (w/v) or 2% (w/v) DSS in drinking water. Clinical endpoints of disease were evaluated 8 days after initiating the DSS treatment. Changes in body weight during the disease progression are shown in **Figure 6**. Weight loss is a reliable method to assess DSS colitis severity; as shown in **Figure 6**, following induction of colitis, $Ido1^{+/+}$ mice exposed to 2% DSS concentration lost weight much more severely than did $Ido1^{-/-}$ mice exposed to 2% DSS concentration ($p < 0.05$, unpaired student's t test). By comparison, at 1% DSS concentration, no significant differences were observed in body weight loss between $Ido1^{-/-}$ and littermate controls (p=not significant). Notably, $Ido1^{-/-}$ mice showed reduced susceptibility to higher concentrations of DSS.

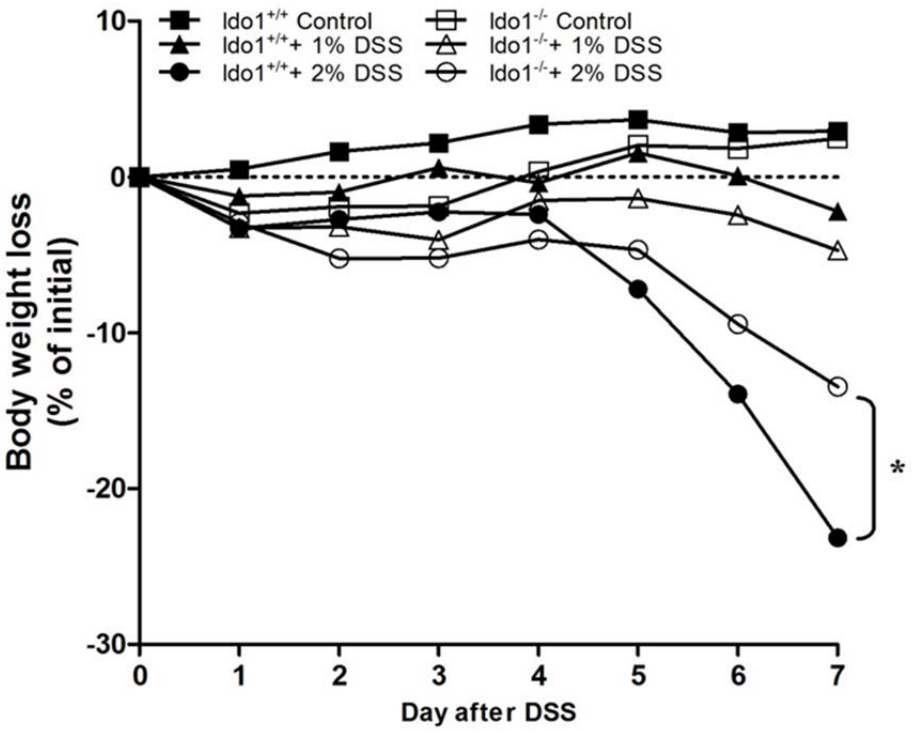


Figure 6. Changes in Body Weight

Body weight curves of Ido1$^{-/-}$ and Ido1$^{+/+}$ mice in an acute model of DSS-induced colitis (weighed daily) for 7 days. Data are presented as the mean of each genotype. (n = 3-5 per group). *P* value were calculated with unpaired student's t test, * $p < 0.05$.

Table 2. Initial and Final Body Weight

	Controls		1% DSS treatment		2% DSS treatment		P value		
	$Ido1^{+/+}$	$Ido1^{-/-}$	$Ido1^{+/+}$	$Ido1^{-/-}$	$Ido1^{+/+}$	$Ido1^{-/-}$	**Genotype effect**	**Dose effect**	**Interaction**
Initial B.W (g)	20.2 ± 0.1	19.4 ± 0.8	21.3 ± 0.2	21.2 ± 0.5	21.1 ± 0.2	20.7 ± 0.3	0.1832	0.0060	0.6458
Final B.W (g)	20.8 ± 0.2 [a]	19.8 ± 0.3 [a]	20.8 ± 0.4 [a]	20.2 ± 0.3 [a]	16.1 ± 0.7 [c]	17.6 ±0.4 [b]	0.8970	<0.0001	0.0207

Data are presented as mean ± SEM (n = 3-5 per group).
[1]Two-way ANOVA was used to determine the main effect (genotype and dose) and interaction.
[2]Different letters indicate significant differences at $P < 0.05$.

Additional endpoints including disease activity index (DAI) over the course of colitis (**Figure 7**, $p < 0.05$ by unpaired student's t test) and colon contraction (**Figure 8**, A and B) also revealed increased disease severity in $Ido1^{+/+}$ mice comparing to $Ido1^{-/-}$ mice ($p < 0.05$ by unpaired student's t test), suggesting that IDO1 produces significant improvements in clinical outcomes of colitis in DSS treated mice.

Histological examination showed that $Ido1^{+/+}$ mice had increased susceptibility to DSS treatment (**Figure 9**). However, there were no obvious differences in histological analysis between $Ido1^{+/+}$ and $Ido1^{-/-}$ in 2% DSS treatment group, since tissue damages were too much severe in both genotypes. The increased damage in DSS-induced $Ido1^{+/+}$ mice correlated with enhanced expression of genes encoding inflammatory markers, including monocyte chemotactic protein-1 (MCP-1), matrix metalloproteinase3 (MMP3), and a transporter associated with antigen processing1 (TAP1) primarily in the colon (**Figure 10**). Such findings suggest that IDO1 exacerbates inflammation in experimental DSS-induced colitis.

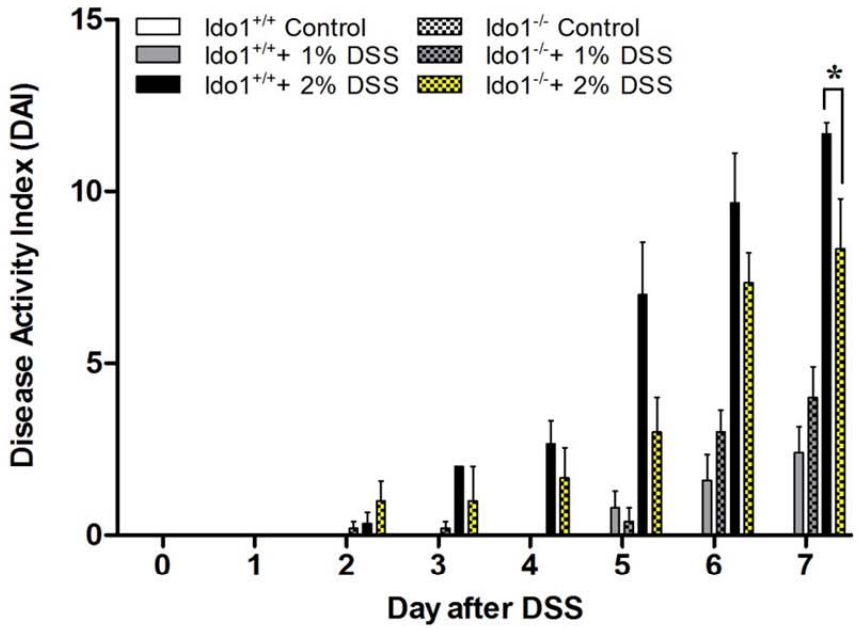
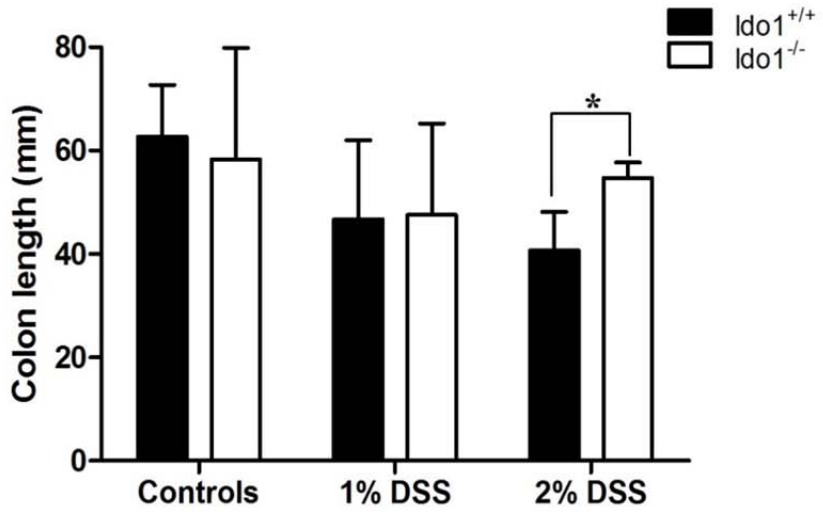


Figure 7. DAI (Disease Activity Index) Score

Stool consistency, fecal bleeding and weight loss were observed on daily basis and DAI (Disease acitivity index) was scored for each mouse. DAI score was graded on a scale of 0-4 as describe in the Materials and Methods. Data are presented as mean ± SEM of each genotype (n = 3-5 per group). Unpaired student's t test was used to determine the significant difference between 2% DSS treatment gorups. *P* value were calculated with unpaired student's t test, Bar with * indicates the significant difference at $p < 0.05$.

A



B

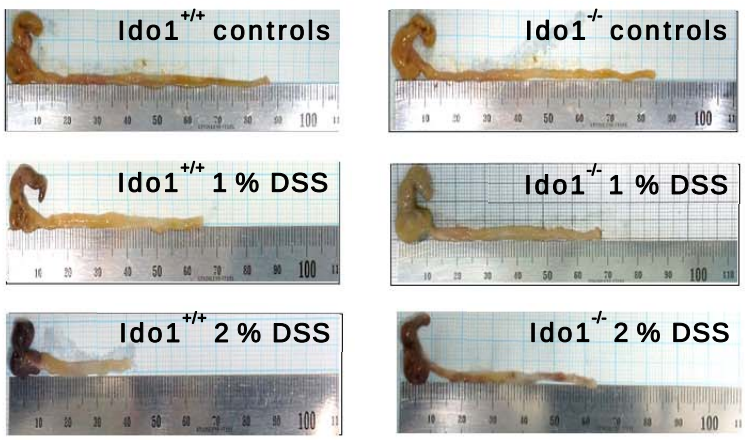


Figure 8. Colon Length (A) and Macroscopic View (B)

(A) Colon length of DSS-induced mice was measured on the 8th day after the start of DSS treatment. Data are presented as mean ± SEM, n=3-5 for each group. Unpaired student's t test was used to determine the significant difference between 2% DSS treatment groups. Bar with * indicates the significant difference at $P < 0.05$ (B) Macroscopic view of the DSS-induced colitis in Ido1$^{+/+}$ and Ido1$^{-/-}$ mice. Representative results from independent mice are shown.

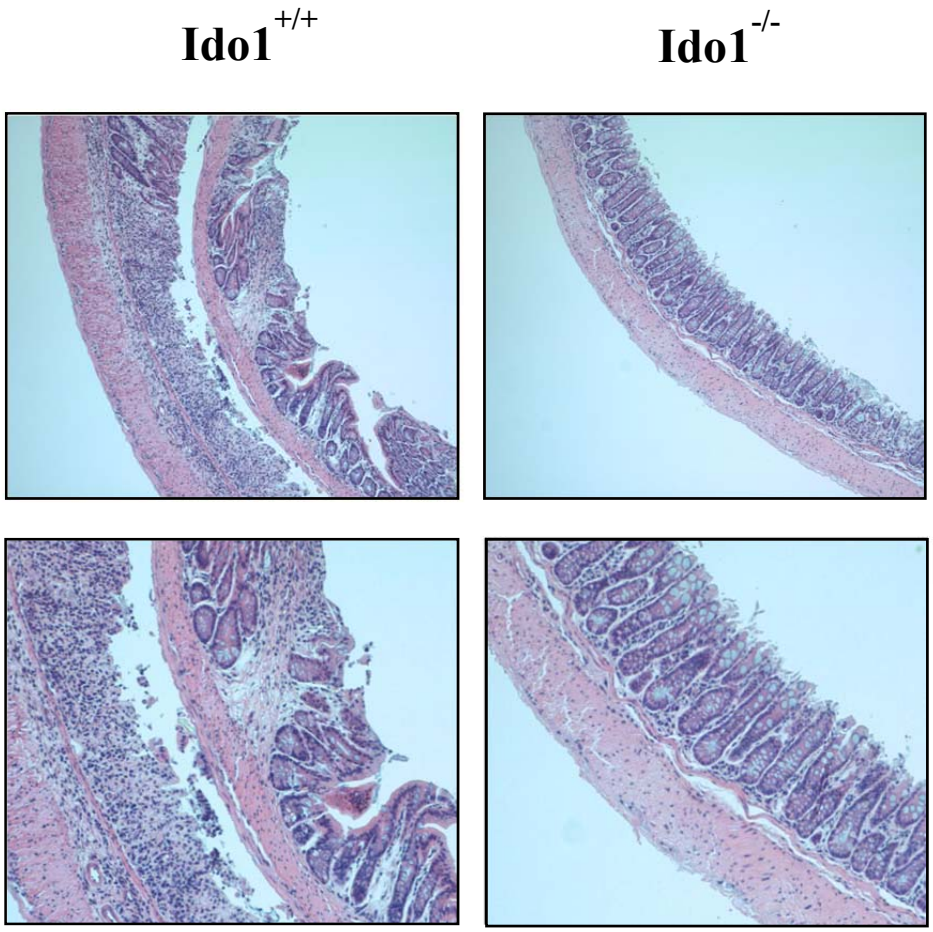
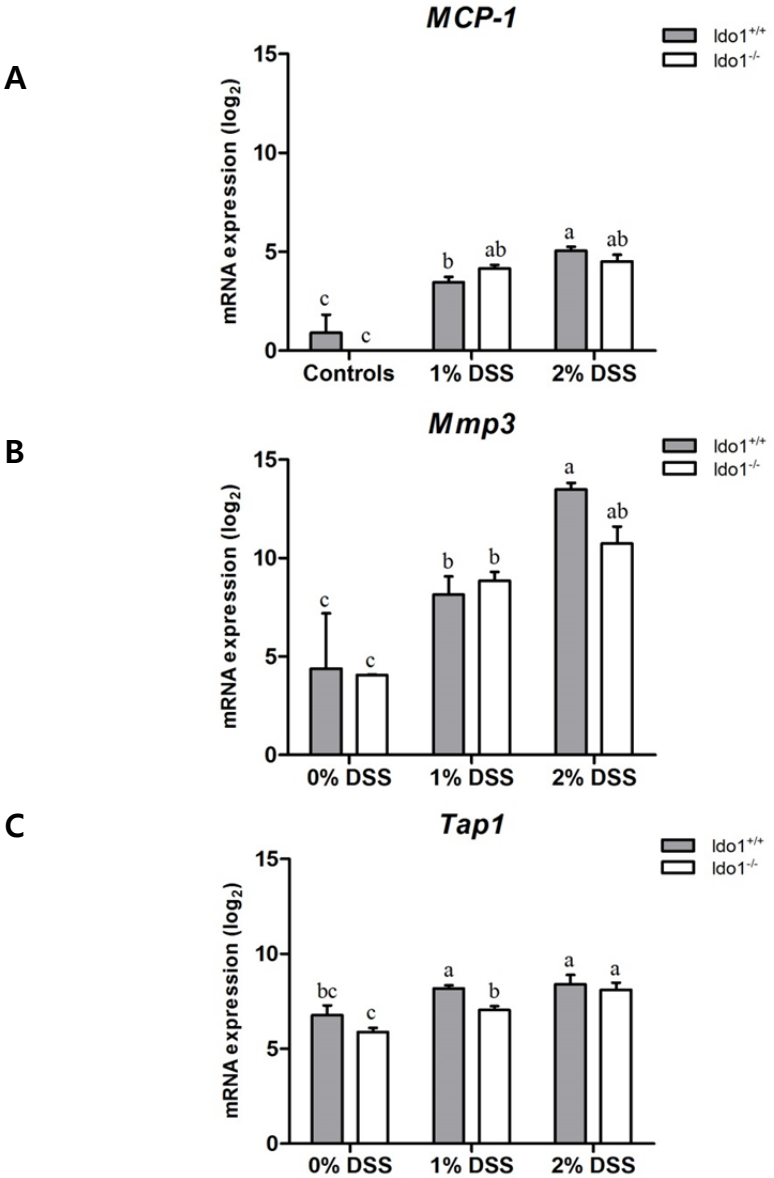


Figure 9. H&E Staining of Colon Tissue Sections

The figure is the representative H&E staining of colon tissue sections (n=3-5 per group, magnification 100X for upper pannels and 200X for lower pannels). Severe mucosal mononuclear cell infiltration and disruption of the normal crypt architecture with epithelial crypt ulceration are observed.



		Mcp-1	*Mmp3*	*Tap1*
***p* value**	**Genotype**	0.4249	0.3760	0.0130
	Dose	<0.0001	<0.0001	0.0002
	Interaction	0.0824	0.2400	0.4114

Figure 10. The mRNA Levels of Genes Involved in Inflammation

The increased damage in DSS-induced Ido1$^{+/+}$ mice correlated with enhanced expression of genes encoding inflammatory markers primarily in the colon. Data are expressed as means ± SEM (n= 3-5 for each group). Bar with different superscripts within the same gene are significantly different at $P < 0.05$. Two-way ANOVA was used to compare with main effect (genotype and dose) and an interaction.

3. IDO1 Regulates the Expression of Multiple Inflammatory Genes in DSS-induced Colitis

To gain insights into the molecular mechanism of the inflammatory bioactivity of IDO1 in this murine model of colitis, transcriptome of DSS-treated $Ido1^{-/-}$ mice was analyzed using microarray, and littermate $Ido1^{+/+}$ mice were used as controls. The RNA samples were reverse transcribed, then hybridized to a Bead Chip Array MouseWG-6 v.2 slide containing about 45,200 oligonucleotides. Data from the microarray were further analyzed using the Genome Studio software. We determined differential gene regulation as a significant result if there were a 2-fold change in expression in biological replicates in comparison with controls. By the 1% false discovery rate in ANOVA test, we identified 5684 genes differentially expressed in the comparison of the six different groups.

First of all, we confirmed the transcript level of IDO1 was significantly increased in inflamed colon tissues, suggesting that IDO1 is associated with inflammatory responses. Additionally, we observed a number of differentially regulated targets caused by IDO1 deficiency in DSS-induced colitis. PCA were used to visualize the overall gene expression patterns from $Ido1^{+/+}$ mice and $Ido1^{-/-}$ mice. We found that $Ido1^{-/-}$ mice had a distinct pattern compared with $Ido1^{+/+}$ mice in the 2% DSS treatment group,

whereas no evident difference was observed between 1% DSS treatment groups (**Figure 11**).

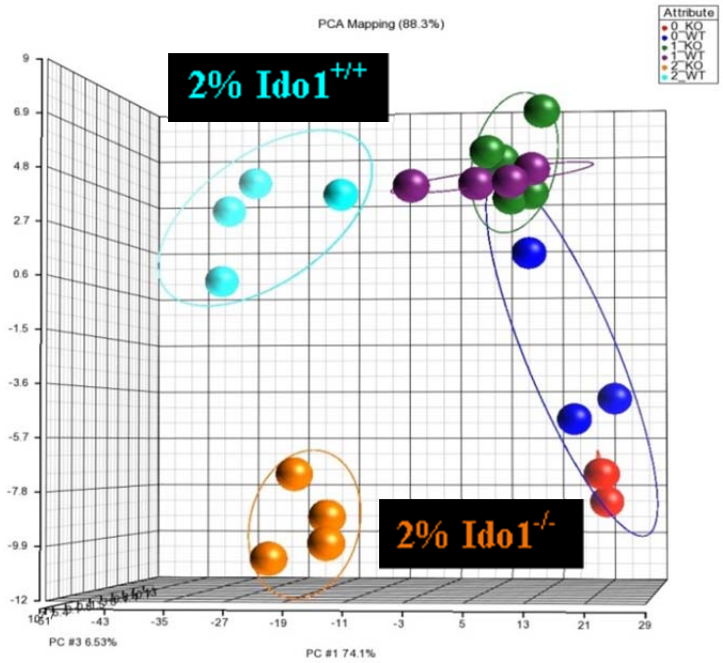


Figure 11. PCA Plot of Transcriptome Analysis

3-D view of PCA scores plot of data obtained on $Ido1^{-/-}$ mice versus $Ido1^{+/+}$ mice treated with DSS. On a 3D-PCA plot of 2% DSS treated $Ido1^{-/-}$ group can be significantly seperated from 2% DSS treated $Ido1^{+/+}$ group. Each spots represents individual mouse in the group. Blue spots, $Ido1^{+/+}$ group ; Red spots, $Ido1^{-/-}$ group; Purple spots, $Ido1^{+/+}$ + 1% DSS treatment group, Green spots, $Ido1^{-/-}$ + 1% DSS treatment group; Sky blue spots, $Ido1^{+/+}$ + 2% DSS treatment group; Orange spots. $Ido1^{-/-}$ + 2% DSS treatment group.

PCA explains overall changes in gene expression profiles, but provide few insights into the biological meaning. To determine the functional relevance of significant genes affected by IDO1 in their gene expressions, we categorized those genes based on their biological functions and tested their significance using IPA. Pathway analysis revealed a large number of regulated genes involved in inflammatory response, cancer, organismal injury, and abnormalities as the key functions that affected IDO1 in $Ido1^{-/-}$ mice (**Figure 12**). We then focused on inflammatory response pathway that was the most significant category modulated by IDO1 deficiency. There were 864 genes in total belonged to inflammatory response category. Specifically, we confirmed inflammation-related networks such as IFN-r, IL-1b, IL-6, toll-like receptors (TLRs) and NF-kB. Our the most striking result was that majorities of members in TLR signaling were down-regulated in their expressions in $Ido1^{-/-}$ mice, suggesting that TLR signaling is essential for roles of IDO1 in DSS-colitis development.

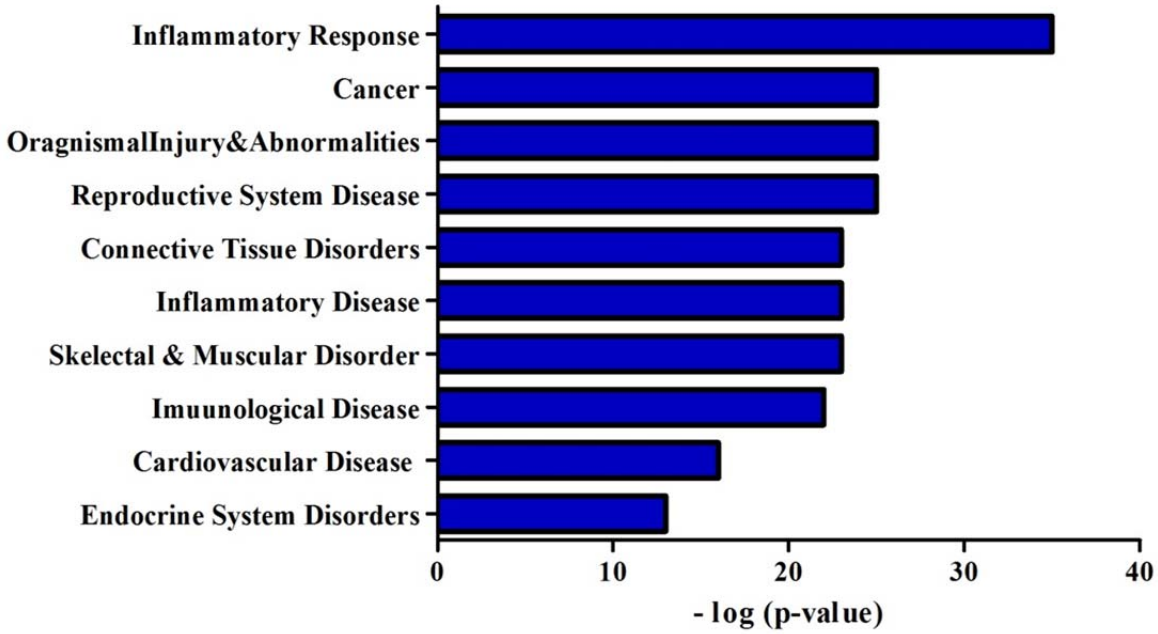


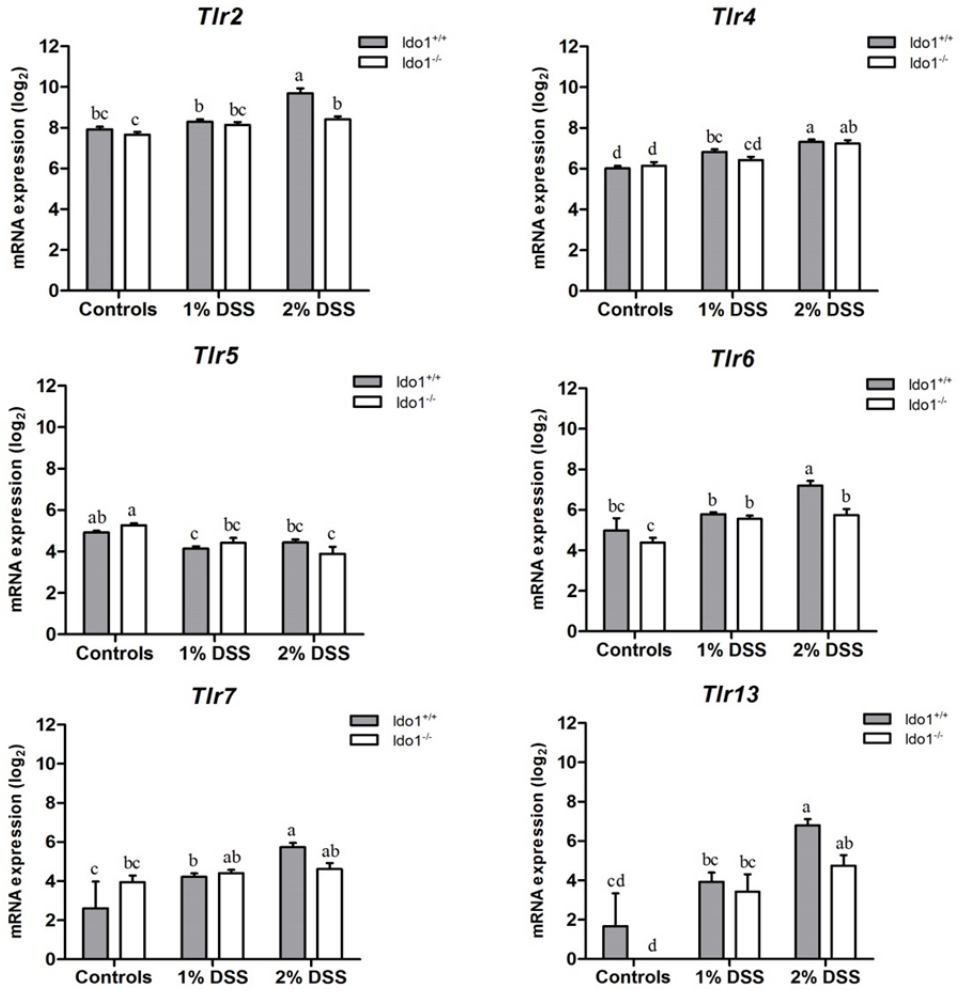
Figure 12. Functional Categories by IPA

Canonical pathway significantly detected in Ido1$^{-/-}$ mice compared to Ido1$^{+/+}$ mice after DSS-induced colitis. Statistical significance of pathway modulation was calculated via a right-tailed Fisher's exact test in Ingenutity Pathway and represented as –log (p value). A larger value on the x axis indicates a higher degree of significance, i.e., a smaller p value. The leading edge analysis of our microarray data identified gene highly correlated with inflammatory response.

4. Absence of IDO1 Resulted in Suppression of TLR Signaling

Members in TLR signaling were dramatically down-regulated in their gene expressions by IDO1 deficiency. Transcript levels of TLR – *Tlr2, Tlr4, Tlr5, Tlr6, Tlr7* and *Tlr13* were significantly reduced ($p<0.05$, one-way ANOVA) in $Ido1^{-/-}$ mice compared to $Ido1^{+/+}$ mice in DSS-induced colitis models (**Figure 13**, A and B). TLRs belong to the family of pathogen-associated pattern recognition receptors and trigger intestinal inflammation when the epithelial barrier is breached by pathogenic microbes. After binding of ligands to TLR triggers a cascade of signaling events via the TLR-complex, downstream molecules are activated and translocated into the nucleus, where they activate transcription factor like c-Fos and c-Jun. We observed c-Jun expression was significantly decreased in $Ido1^{-/-}$ mice compared to $Ido1^{+/+}$ mice.

A



p value		*Tlr2*	*Tlr4*	*Tlr5*	*Tlr6*	*Tlr7*	*Tlr13*
	Genotype	0.0006	0.3553	0.8772	0.0026	0.7282	0.0388
	Dose	<0.0001	<0.0001	0.0013	<0.0001	0.0044	<0.0001
	Interaction	0.0044	0.2427	0.093	0.0657	0.0644	0.5389

Figure13A. Differential Expression of a Selection of Genes Associated with TLR signaling

Differential expression of a selection of genes associated with TLR signaling (*Tlr 2,4,5,6,7,13*) in Ido1$^{-/-}$ *versus* Ido1$^{+/+}$ mice. Data are presented as means ± SEM (n = 3-5 for each group). Bar with different superscripts within the same gene are significantly different at $P < 0.05$. Two-way ANOVA was used to compare with main effect (genotype and dose) and an interaction.

B

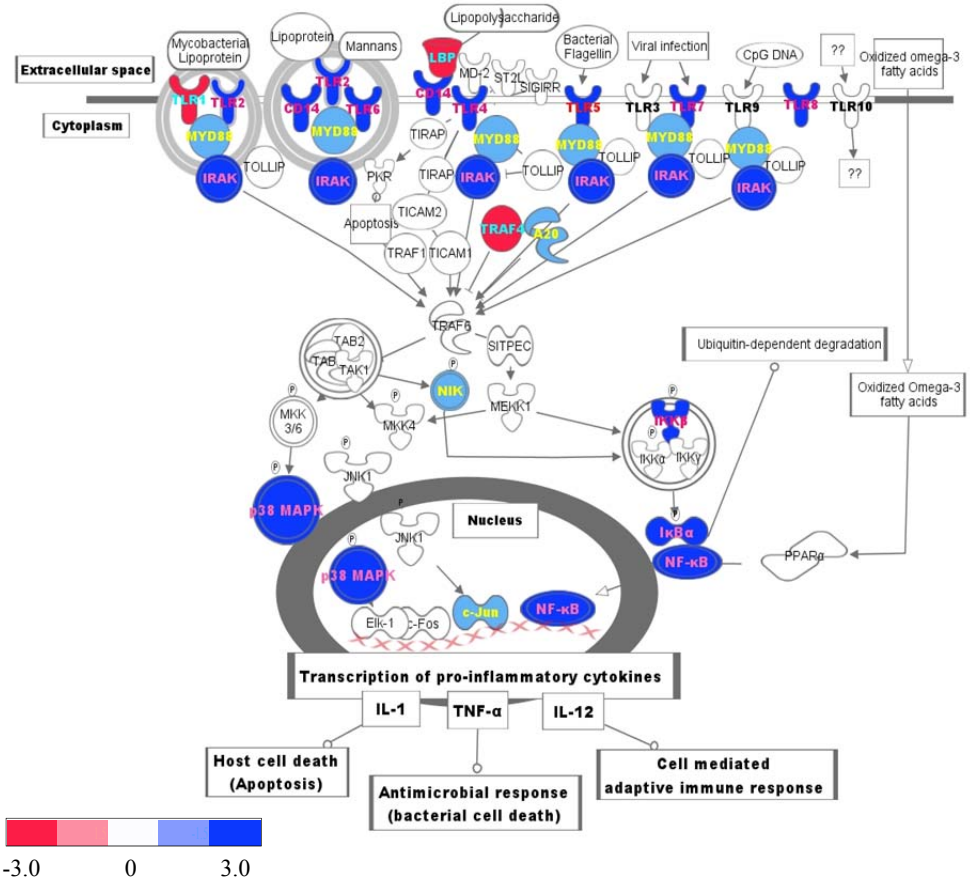
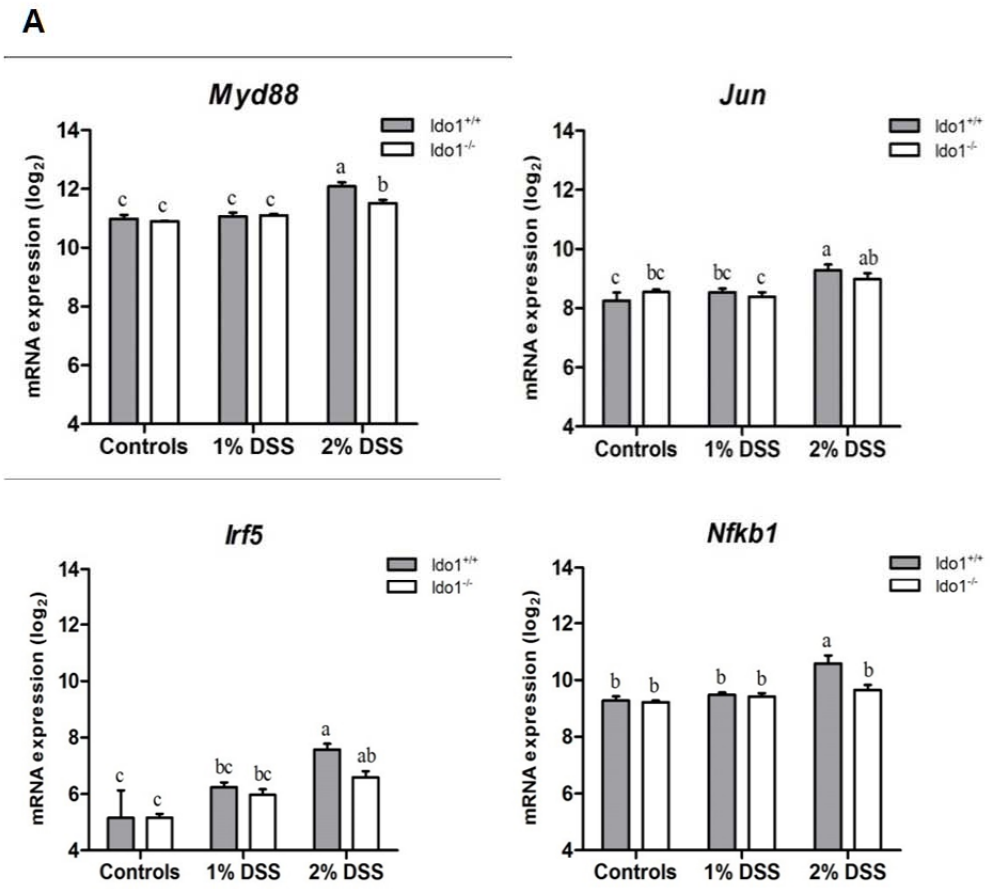


Figure13B. Pathway Model of TLR Signaling Based IPA Knowledge
Pathway model of TLR signaling based IPA knowledge. Genes in blue color indicate down-regulation and in red color indicate up-regulation.

In this study, we also ascertained that TLR triggered cascades downstream signaling molecules like MyD88 (myeloid differentiation primary response gene 88), p38 MAPK (p38 map kinase), JNKs (JUN N-terminal kinases), IRF5 (interferon regulatory factor 5), and NF-kB (nuclear factor kB) are down-regulated (**Figure 14**, A and B). All TLRs except TLR3 signal through MyD88-dependent pathway. TLR3 signals only through MyD88-independent TRIF-dependent pathways, while TLR4 is distinct as it can signal through both MyD88-dependent as well as MyD88-independent pathways (Shikha Tarang, 2012). MyD88, a key adaptor protein in the signal transduction cascades, which shared most of the TLRs, in this study, MyD88-dependent TLR signaling pathways have been identified (**Figure 13B**), suggesting that the differences in colitis development status between $Ido1^{-/-}$ and $Ido1^{+/+}$ mice described in previous sections are resulted from dysregulation of TLR-MyD88 signaling pathway. For the further understanding of the molecular mechanisms regulating the expression of TLRs, we investigated the mechanism by which IDO1-deficiency down-regulates TLRs expression.



p value		*MyD88*	*Jun*	*Irf5*	*Nfkb1*
	Genotype	0.0341	0.7390	0.1560	0.0176
	Dose	<0.0001	0.0011	0.0002	0.0001
	Interaction	0.0265	0.3200	0.3726	0.0196

Figure14A. Differential Expression of a Selection of Genes Associated with NF-kB Signaling

Differential expression of a selection of genes associated with NF-kB signaling (*Myd88, Jun, Irf5, Nfkb1*) in Ido1$^{-/-}$ *versus* Ido1$^{+/+}$ mice. Data are presented as means ± SEM (n= 3-5 for each group). Bar with different superscripts within the same genes are significantly different at $P < 0.05$. Two-way ANOVA was used to compare with main effect (genotype and dose) and an interaction.

B

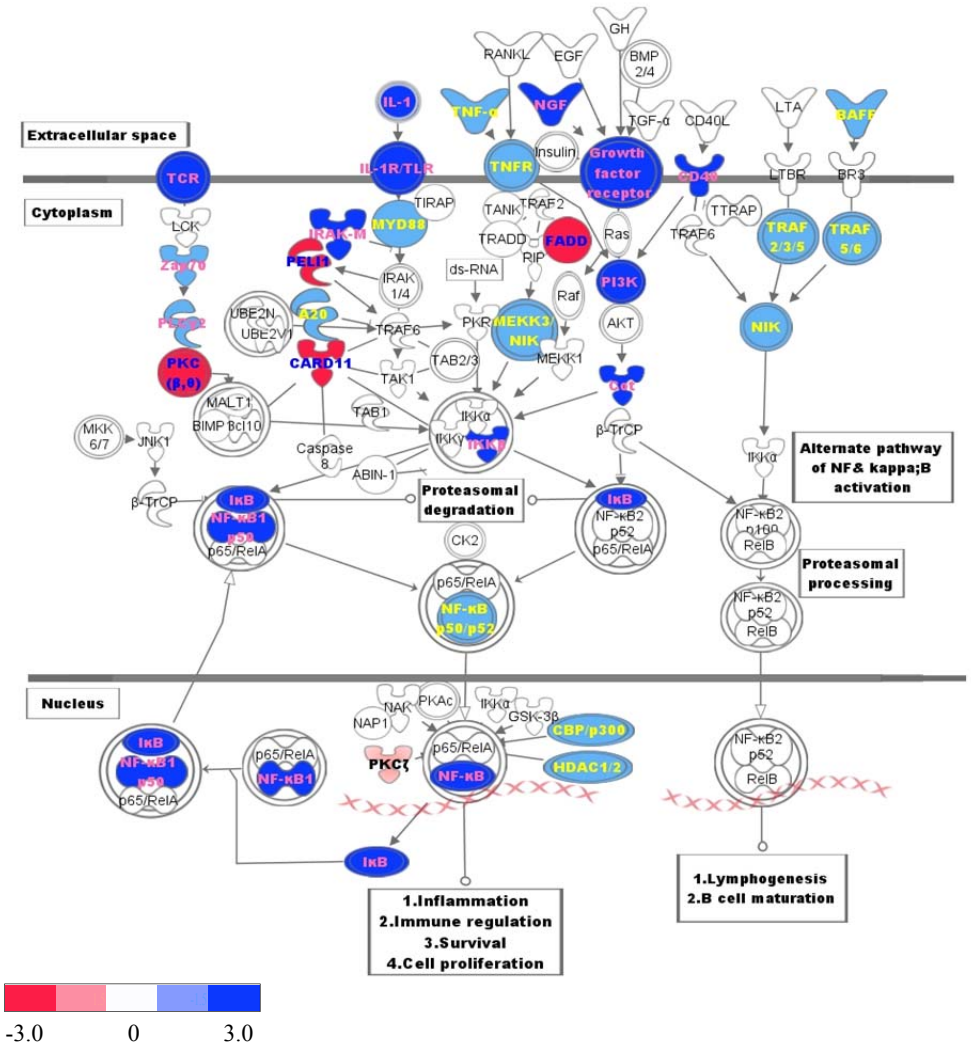


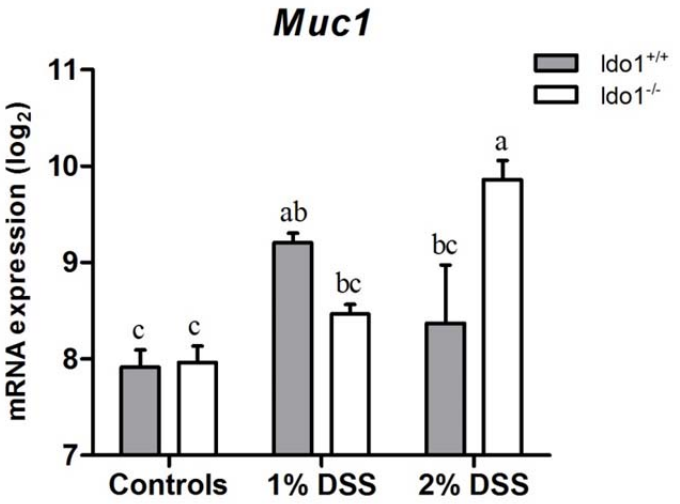
Figure14B. Pathway Model of NF-kB Signaling Based IPA Knowledge
Pathway model of NF-kB signaling based IPA knowledge. Genes in blue color indicate down-regulation and in red color indicate up-regulation.

5. MUC1 Suppresses TLR Signaling

A number of studies reported intestinal tissues in colitis had impaired and permeable mucosal barriers. Mucin is the first line of host defenses against invading bacteria, and its alteration severely affects epithelial barrier function. Increased bacterial invasion and translocation primarily depend upon depletion of the thick mucin layer or mucin wash out due to mucosal inflammation. Therefore, to determine whether the enhanced inflammatory response in colon tissue of $Ido1^{+/+}$ mice was due to impaired host defense in these mice, we assessed the gene expression of Muc1 that has been up-regulated for defense activity in colitis. A basal low-level expression of Muc1, which was a similar level in $Ido1^{-/-}$ and $Ido1^{+/+}$ mice, significantly increased in $Ido1^{-/-}$ mice during inflammatory stimuli compared to $Ido1^{+/+}$ controls (**Figure 15**).

This data suggests that IDO1 markedly affected mucin release to restrict the mucosal damaging effects of DSS, such as mucus depletion and crypt inflammation. There are several studies on the regulation of TLRs by Muc1 where TLRs were suppressed by Muc1 *in vivo* and *in vitro* (Ueno et al., 2008; Williams et al., 2009). The evaluated levels of Muc1 under inflammatory conditions lessen inflammation by inhibiting further activation of TLR signaling (Kato et al., 2009; Tallant et al., 2004). These

results suggest that expression of Muc1 may be therapeutically important for maintenance of normal epithelial barriers in the intestine.



P value		
Genotype	**Dose**	**Interaction**
0.2674	0.0030	0.0020

Figure15. Colonic mRNA Expression Level of Muc1 Genes after DSS Treatment

Data are presented as means ± SEM of each genotype (n= 3-5 per group). Bars with different superscripts within the same gene are significantly different at $P < 0.05$. Two-way ANOVA was used to compare with main effect (genotype and dose) and an interaction.

6. Inflammatory Cytokines and Chemokines Production in Colon is Affected by IDO1

The inflammatory role of IDO1 was dependent on the TLR-MyD88 signaling pathway as above results. TLR-triggered molecules further lead to the production of pro-inflammatory cytokines (**Figure 13B**) and chemokines, which activate adaptive immunity and mount an effective immune response against invading pathogens (Miggin et al., 2006). Binding of ligands to TLRs initiate a complex signaling cascade leading to the activation of several pro-inflammatory cytokines, which via autocrine and paracrine effects, initiates a cascade of intracellular signaling. In addition, MyD88 deficiency abrogates or delays pro-inflammatory responses to various TLR ligands including LPS, peptideglycan, flagellin, and CpG (Takeda et al., 2003). Therefore, we investigated the *in vivo* effects of IDO1 on inflammatory responses and production of local inflammatory cytokines mechanistically linked to inflammatory-related colonic injuries in DSS-induced colitis.

The gene expression profiles of cytokine and chemokine in colon tissues of DSS-treated $Ido1^{-/-}$ mice and their littermate controls were analyzed (**Figure 16**).

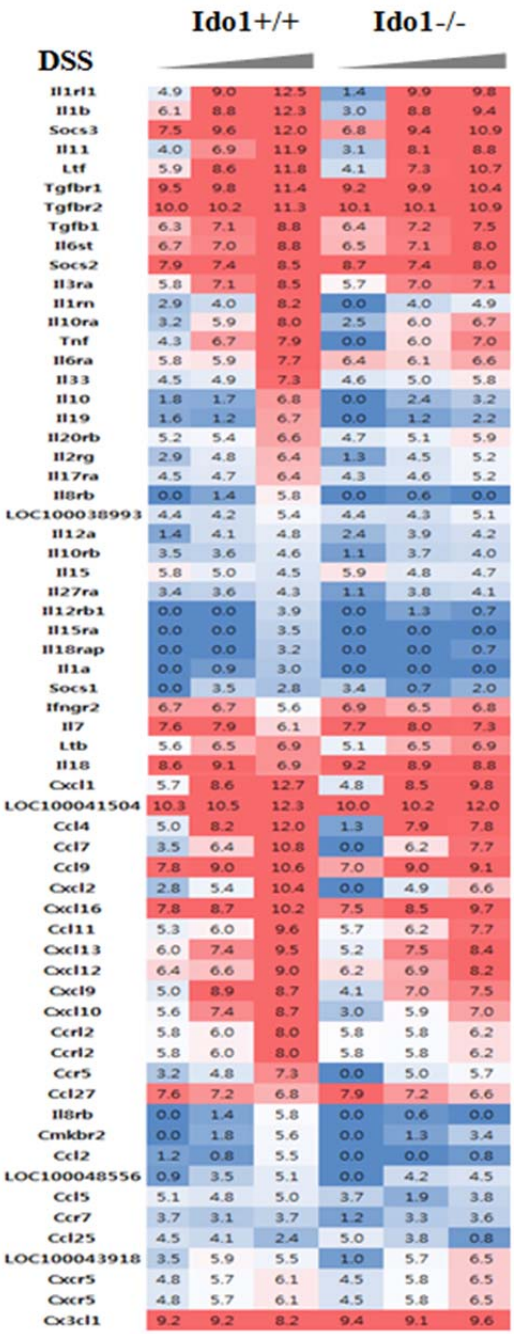
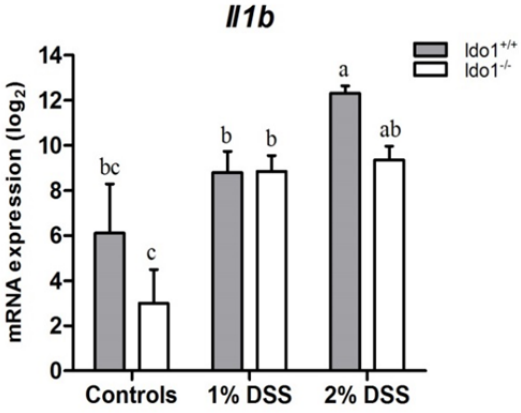


Figure16. Expression Profiling of Inflammatory Cytokines and Chemokines in Colon Tissue after DSS Treatment

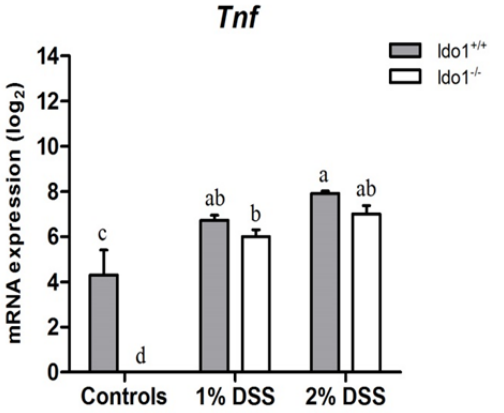
Heat map of cytokines and chemokines genes that are differentially expressed in $Ido1^{-/-}$ *versus* $Ido1^{+/+}$. One-way ANOVA was used to determine the significant difference among the groups. The given values are the average of normalized intensities (3-5 mice per group)

mRNA expressions of pro-inflammatory cytokines including *Il1b, and Tnf,* central mediators of inflammation, were elevated in colonic tissue of $Ido1^{+/+}$ *versus* $Ido1^{-/-}$ mice (**Figure 17A**). The expressions of the anti-inflammatory cytokines including *Il7* and *Il18* mRNAs were decreased in $Ido1^{+/+}$ mice (**Figure 17B**). These results implied that TLRs signaling was inhibited by IDO1-deficiency, which suppressed pro-inflammatory cytokines and chemokines production through the regulation of a multitude of transcription factors such as NF-kB in the DSS-induced colitis.

A

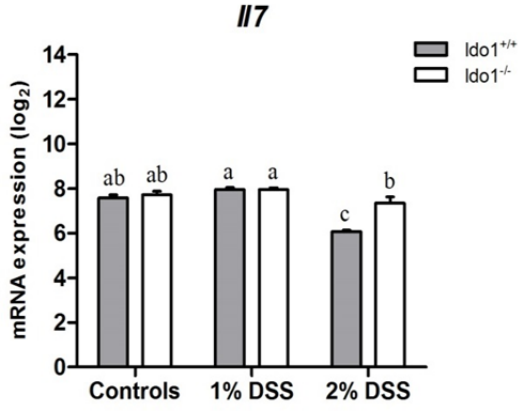


P value		
Genotype	Dose	Interaction
0.0310	<0.0001	0.2218

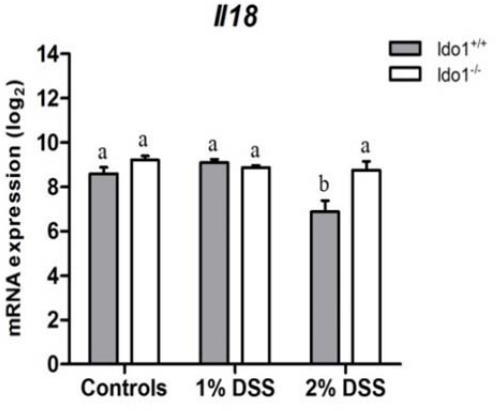


P value		
Genotype	Dose	Interaction
<0.0001	<0.0001	0.0010

B



P value		
Genotype	Dose	Interaction
0.0011	<0.0001	0.0006



P value		
Genotype	Dose	Interaction
0.0068	0.0013	0.0055

Figure17. The mRNA Levels of the Genes Related to Pro-inflammatory (A) or Anti-inflammatory (B) Cytokines in Colon Tissue

Data are presented as means ± SEM, n=3-5 for each group. Bar with different superscripts within the same gene are significantly different at $P < 0.05$. Two-way ANOVA was used to compare with main effect (genotype and dose) and an interaction.

7. Inflammation and Tryptophan Metabolism

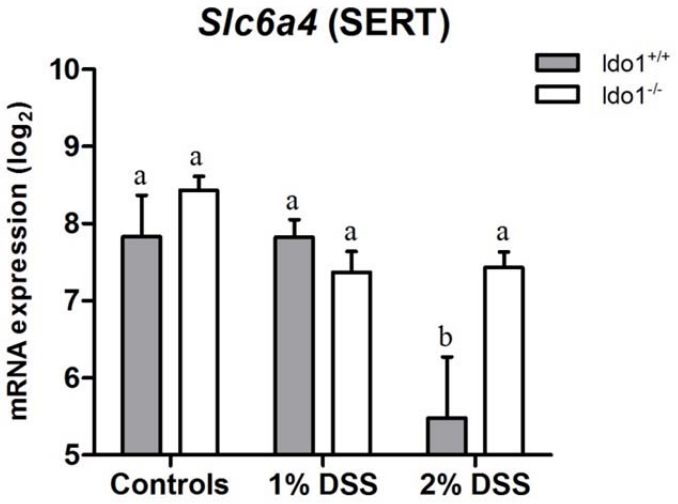
We hypothesized that Ido1$^{-/-}$ mice were relatively inefficient at initiating tryptophan metabolism, resulting in impaired IDO1 activity, which might reduce the production of tryptophan metabolites like kynurenine and increase free tryptophan. The inactivated kynurenine pathway may further activate the serotonin pathway, increasing serotonin synthesis from its precursor-free tryptophan.

Serotonin is synthesized in neurons of the central nervous system; however, interestingly, the vast majority of serotonin was localized in the intestine (Levin et al., 2013). As serotonin is bio-synthesized in the intestine, it has been reported to play a role in the pathophysiology of IBD and colitis (Levin et al., 2013; De Abajo et al., 1999). A previous study reported that deviant intestinal serotonin signaling pathway that occurs in the pharmacological inhibition or genetic loss of SERT, the major regulator of serotonin levels in tissue, leads to abnormal gastrointestinal motility by an alternating pattern of diarrhea and constipation, and aggravates inflammation of the colon (Manocha et al., 2012). In the TNBS-induced colitis mouse model, lack of SERT to investigate the role of serotonin signaling in intestinal inflammation enhances the severity of the colitis, suggesting that the evaluated endogenous serotonin promotes lymphocyte activation and exerts a pro-inflammatory effect by pro-inflammatory

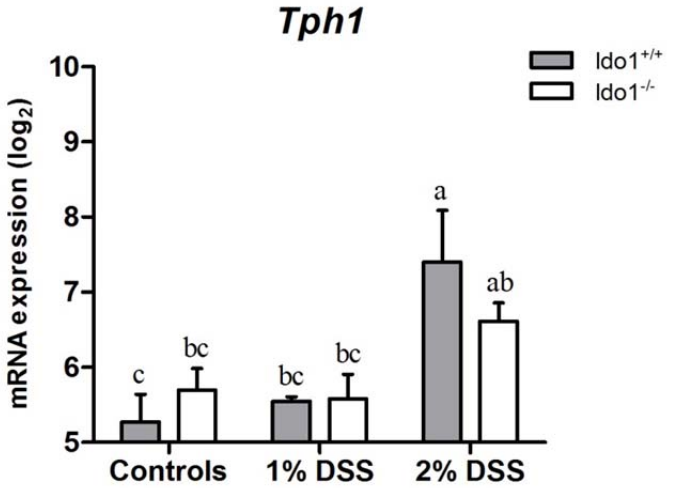
cytokines in the colon (Linden et al., 2003).

In the present study, we confirmed the role of serotonin generated by IDO1 deficiency in regulating intestinal inflammation in $Ido1^{-/-}$ mice treated by DSS. The differences in the colitis in $Ido1^{-/-}$mice and $Ido1^{+/+}$ mice is due to the low serotonin content of the colon by normal SERT activity which is required to removal of serotonin from its extracellular receptor. Transcripts encoding SERT were quantified in the colon from each group of mice. Inflammation possibly increased the tryptophan uptake, and led to down-regulation of SERT with a coincident increase in the availability of serotonin release. Our result showed that the abundance of transcripts encoding SERT is up-regulated over that in $Ido1^{-/-}$ compared to controls (**Figure 18**). An increase in SERT expression would reduce the pro-inflammatory effects of serotonin, and decrease the severity of inflammation. It is possible to inhibit intestinal mucosal serotonin signaling selectively and suppress intestinal inflammation.

To find out the correlation with Ido1 deficiency and the conversion of tryptophan to serotonin, mRNA expression of *Tph1*, the key enzyme for serotonin biosynthesis, was examined. The mRNA expression of *Tph1* was down-regulated in $Ido1^{-/-}$ mice (**Figure 18**). Previous findings have reported an attenuated DSS-induced colitis in $TPH^{-/-}$ mice compared to $TPH^{+/+}$ mice, suggesting a low serotonin level accompanied with down-regulation of IL-17 and IFN-r levels in colonic tissues (Li et al., 2011). We also investigated gene expression which was involved in metabolic pathways of tryptophan in IDO1 downstream, as shown in Figure 19A, under inflammatory conditions (**Figure 19**, B and C). Modulation of tryptophan metabolism, especially serotonin signaling pathway play a key role, and be a novel target in the intestinal inflammation.



P value		
Genotype	**Dose**	**Interaction**
0.0599	0.0039	0.0241



P value		
Genotype	**Dose**	**Interaction**
0.7230	0.0009	0.3047

Figure18. The mRNA Levels of Genes Related to Serotonin Signaling in Colon Tissue

Data are presented as means ± SEM, n=3-5 for each group. Bars with different superscripts within the same gene are significantly different at $P < 0.05$. Two-way ANOVA was used to compare with main effect (genotype and dose) and an interaction.

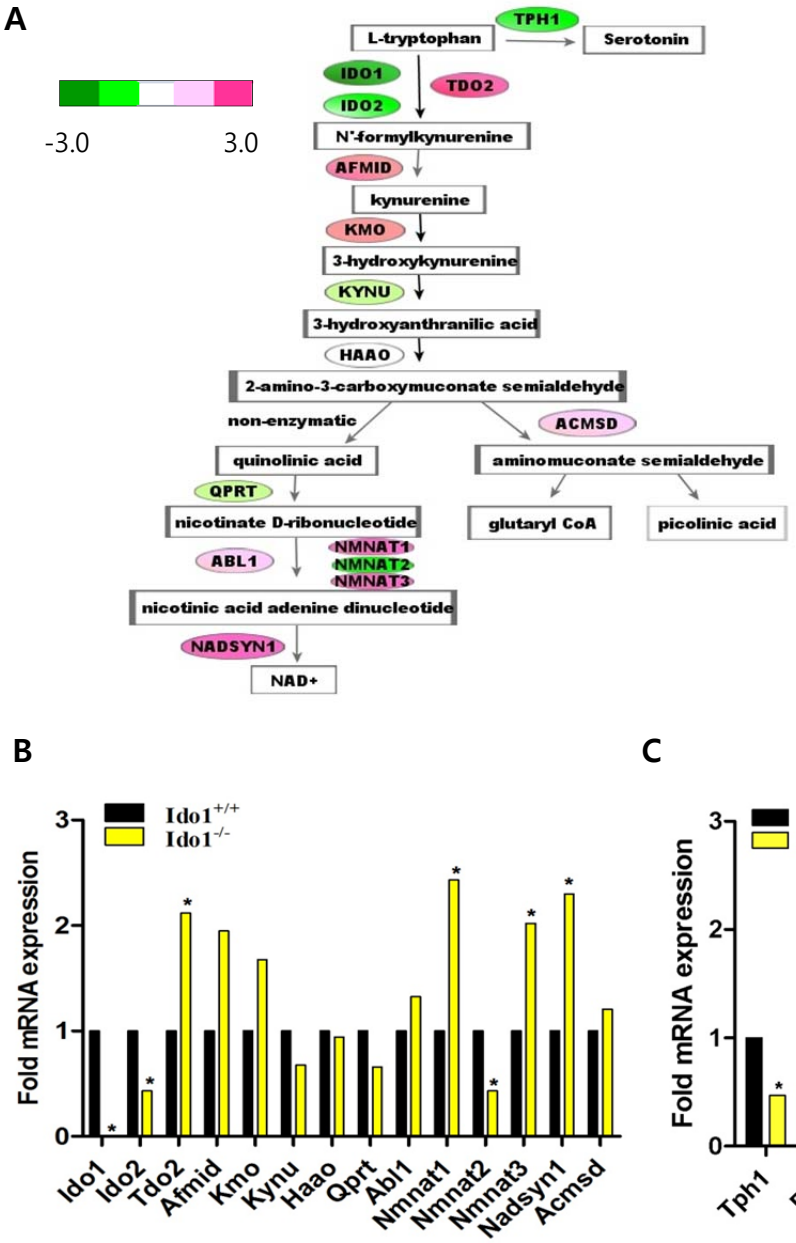


Figure19. The mRNA Levels of Genes Related to Tryptophan Metabolism in Inflammatory Condition

(A) The major metabolic pathway of tryptophan in the inflammatory condition (B) Differential expression of a selection of genes from (A) in the colon tissue was determined by quantification of fold mRNA in DSS-treated $Ido1^{-/-}$ and $Ido1^{+/+}$ littermates where the values for $Ido1^{+/+}$ mice were set 1.0. Bar with * indicate significant difference at $P < 0.05$ by unpaired student t test or > 2-fold at least one comparison.

Discussion

Tryptophan metabolism within various organ tissues is linked with numerous biological and physiological functions. Tryptophan has critical metabolic functions for the regulation of growth, mood, cognition, behavior, and immune response (Richard et al., 2009). Dietary tryptophan is primarily absorbed via the transport pathway on the luminal surface of small intestinal epithelial cells. In particular, gut intestine which has higher IDO1 expression than other tissues (Matteoli et al., 2010) regulates tryptophan homeostasis through degrading excess tryptophan.

To determine the roles of IDO1 at baseline in the intestine, the gene expression profiles were analyzed with an IDO1-deficient mouse model. It was found that genes significantly differentially expressed by IDO1 were strongly associated with inflammatory response, demonstrating a key role for this enzyme mediating inflammatory response in the intestine. This is particularly important in the intestine because of the various microorganisms and food antigens which challenge the intestinal immune system. As the luminal surface of the gastrointestinal tract continually interacts with foreign antigen such as pathogenic bacteria, the mucosal immune system maintains immune tolerance to limit inflammatory

responses against the antigen attacks. When tolerance has its negative tradeoffs, it allows some pathogenic microbes to infect a host immune system. Impaired immune tolerance in the intestine can lead to inflammatory bowel disease (IBD) like Crohn's disease (CD) and ulcerative colitis (UC) (Zhou et al., 2012; Wolf et al., 2004).

In the present results, consistent with previous studies, IDO1 expression was induced in many intestinal inflammatory conditions of wild-type mice (Gurtner et al., 2003), and remarkably exacerbated the inflammation in experimental DSS-induced colitis. The underlying mechanism of the roles of IDO1 in colitis has been a matter of debate. Opposing result was been reported by other researchers using the same model (Takamatsu et al., 2013). In the trinitrobenzene sulfonic acid (TNBS) colitis model, the inhibition of IDO1 exacerbated colitis due to down-regulating regulatory T cell responses within intestinal tract (Takamatsu et al., 2013; Chen et al., 2008). This result suggested that IDO1 had therapeutic properties to limit colitis severity. To elucidate the role of the enzyme in this colitis model in non-T cell mediated mechanism, acute DSS-induced colitis experiment mouse model was selected rather than TNBS colitis.

In this study, we have shown that the IDO1 contributes to DSS-treated colitis. Five marked differences between $Ido1^{+/+}$ mice and $Ido1^{-/-}$ mice with the DSS-induced model were founded as follow. 1) Disease progression:

IDO1 may cause colonic inflammation early on after DSS treatment. IDO1 expression and enzymatic activity are related with disease activity. 2) TLRs-MyD88 signaling dependence: IDO1 may require TLRs-MyD88 signaling to cause colitis. 3) Muc1 regulates TLRs signaling: strains with disrupted Ido1 may elevate the level of Muc1 mRNA expression which reduce the inflammation by inhibiting further activation of TLRs signaling. 4) Pro-inflammatory cytokines and chemokines production dependence: IDO1 affects inflammatory cytokines and chemokine production to cause colitis. 5) Serotonin signaling pathway dependence: IDO1 regulates serotonin signaling to cause colitis.

These observations supported that the function of IDO1 in intestinal tissues regulates the balance between inflammation and regulatory responses in the intestine. Especially, there were striking results indicating that disrupted TLR signaling was attributable to the attenuation of DSS-induced colitis in $Ido1^{-/-}$ mice. In addition, TLR-triggered cascades of downstream mechanism molecules like JUN and NF-kB signaling are also directly down-regulated; as a result, the release of pro-inflammatory cytokines such as Il-1b and Tnf were decreased. This result suggested that IDO1 is a key enzyme which mediates by linking the innate and adaptive immune responses.

Tryptophan degradation via kynurenine pathway by IDO1 has been explored as a crucial regulator of the immune response. IDO1 has been

reported to induce by the pro-inflammatory cytokine interferon-r (IFN-r) in APCs, and express in inflamed tissue (Takamatsu et al., 2013). For example, low level of tryptophan might be detected by the mTOR or stress responsive kinase GCN2, both of which respond to low levels of amino acids. GCN2 has been reported to be necessary for the immunoregulatory effects of IDO1 in T cells (Munn et al., 2005). These important functions of IDO1became an emerging target for cancer, infection, autoimmunity, and other diseases characterized by inflammation conditions and immune suppression (Mellor et al., 2004; Platten et al., 2012; Klonowski et al., 2012; Blumenthal et al., 2012). If so, how does IDO1 regulated uptake of tryptophan in the small intestine affect DSS-induced inflammation in the intestine?

In our analysis, both mRNA expression of reduced SERT (which terminates serotonin reuptake) and elevated Tph1 (biosynthesizes to serotonin) was found to increase the level of serotonin in the intestine. The regulation of intestinal inflammation by modulating NF-kB activation and the production of pro-inflammatory cytokines from innate immune cells were found to be affected by serotonin (Li et al., 2011).

There is also emerging evidence that serotonin signaling plays an important role in inflammation-induced physiological changes, including mucin secretion and immunoregulatory effects in the intestine (Keszthelyi et al., 2012; Khan et al., 2010; Gjia et al., 2009; Bischoff et al., 2009; Coates et

al., 2004). Although serotonin has been demonstrated to be involved in immune system function, its precise role remains unclear.

More recently, metabolites of the kynurenine have been considered to play a regulatory role in the CNS and the gastrointestinal tract (Stone et al., 2013). The biologic relevance of IDO1 has been reported in the models of inflammation associated with diverse diseases (Zwilling et al., 2011; Schwarcz et al., 2012). The metabolic intermediates such as kynurenine and quinolinic acid could have a physiological role as agonists or antagonists at various receptor sites. They could inhibit proliferation of T cells, and induce the apoptosis of activated T cells (Kim et al., 2010). However, relatively little information is available regarding the response of tryptophan metabolites, and the intricate machinery evolved for the regulatory role of tryptophan metabolites in the colon that could still be unknown. Indeed, although some tryptophan metabolites have potential palliative properties (Pero et al., 2009), some other endogenous tryptophan metabolites are known to have toxicity which increases an oxidative stress (Sahm et al., 2013). These contradictory results revealed the complexity of tryptophan metabolism and the various properties of tryptophan metabolites. Therefore, further studies are needed to investigate the complexity of tryptophan metabolism and its regulation at each level of tryptophan metabolites in the intestine.

The present report may offer the first comprehensive description of the role of the IDO1 at the transcriptome level using a DSS-treated Ido1$^{-/-}$ mouse model, and describe new molecular targets which specifically expressed or restricted a tryptophan metabolism in relation to colon inflammation through a global profiling approach. This may be a novel target of therapeutic intervention in colitic disease.

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국문초록

Ido1 knock-out 마우스를 이용한 대장염 모델에서의 전사체 분석

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트립토판은 인간을 비롯한 대부분의 동물들에서 합성되지 않으므로, 반드시 식이를 통해 섭취해야만 하는 필수 아미노산이다. 트립토판은 다양한 생물학적 과정에서 전구체로서 사용되거나, 첫 번째 속도 제한 효소인 IDO1(Indoleamine 2,3-dioxyganase)에 의해 Kynurenine 과 같은 대사 물질로 대사될 수 있다. IDO1 은 뇌, 비장, 폐 등 인체 내 여러 장기에서 발현되는데, 특히 장 조직에서의 발현이 가장 높다. 그러나 실제 장 내에서 IDO1 이 하는 역할과 기능에 관한 연구는 미흡한 실정이다. 따라서 본 연구는 대장 내 IDO1 의 생체 내 역할 및 기능을 규명하고자 $Ido1^{-/-}$ 마우스와 $Ido1^{+/+}$ 마우스에서 IDO1 에 의하여 발현이 조절되는 유전자들을 microarray 실험을 통해 탐색하였다. $Ido1^{-/-}$마우스 및 $Ido1^{+/+}$ 마우스 두 그룹간 발현이 유의하게 변한 유전자들을 screening 한

결과, 총 102 개의 유전자들의 발현이 유의적으로 변화하였다. 발현이 변한 유전자들에 대한 생물학적 기능을 탐색하고자, Gene Set Enrichment Analysis 를 수행한 결과, 염증 반응(Inflammatory response)에 관여하는 유전자들이 대부분 유의적으로 변했음을 확인하였다. 이러한 결과를 바탕으로 염증성 장 질환 모델에서 IDO1 의 기능 및 역할을 알아보고자 하였다. 10 주령의 암컷 C57BL/6J wild-type ($Ido1^{+/+}$) 마우스와 $Ido1^{-/-}$마우스를 이용하여 1% 및 2% DSS(Dextran Sulfate Sodium) 음수를 공급하여 대장염 모델을 유도하였다. 체중, DAI(Disease Activity Index, 설사 · 혈변 점수), 장 길이, 조직학적 손상 정도(H&E 염색), 염증성 세포 수 등을 확인하였을 때, $Ido1^{-/-}$ 마우스에서 $Ido1^{+/+}$마우스에 비해 질병의 진행 정도나 속도가 유의적으로 낮은 것으로 관찰되었다. 대장염의 발달 및 진행 속도에 기여하는 유의한 신호전달 경로를 탐색하기 위하여, 염증이 생긴 대장 조직으로부터 total RNA 를 추출하여 microarray 실험을 수행하였다. 그 결과 두 유전자형 사이에 질병의 진행 차이를 발생시키는데 기여하는 신호 전달 경로가 탐색되었다. $Ido1^{-/-}$ 마우스에서 $Ido1^{+/+}$마우스에 비해 TLR signaling 및 NF-kB signaling 에 관여하는 대부분의 유전자들의 발현이 매우 유의적으로 감소했음을 확인할 수 있었다. 뿐만 아니라 $Ido1^{-/-}$ 마우스에서 많은 염증성 (pro-inflammatory) 및 항염증성 사이토카인 (anti-inflammatory cytokine) 및 케모카인들의 발현이 변화됨이 관찰되었다. 위 결과들을 종합하였을 때, IDO1 은 장 내 염증 반응과 전사조절 네트워크를

변화시킴으로써, 대장염 유도 및 진행에 있어 주요 인자로 작용하는 것으로 제안된다.

주요어: 트립토판, IDO1(Indoleamine 2,3-dioxygenase), 대장염, 전사체, microarray, 염증 반응

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